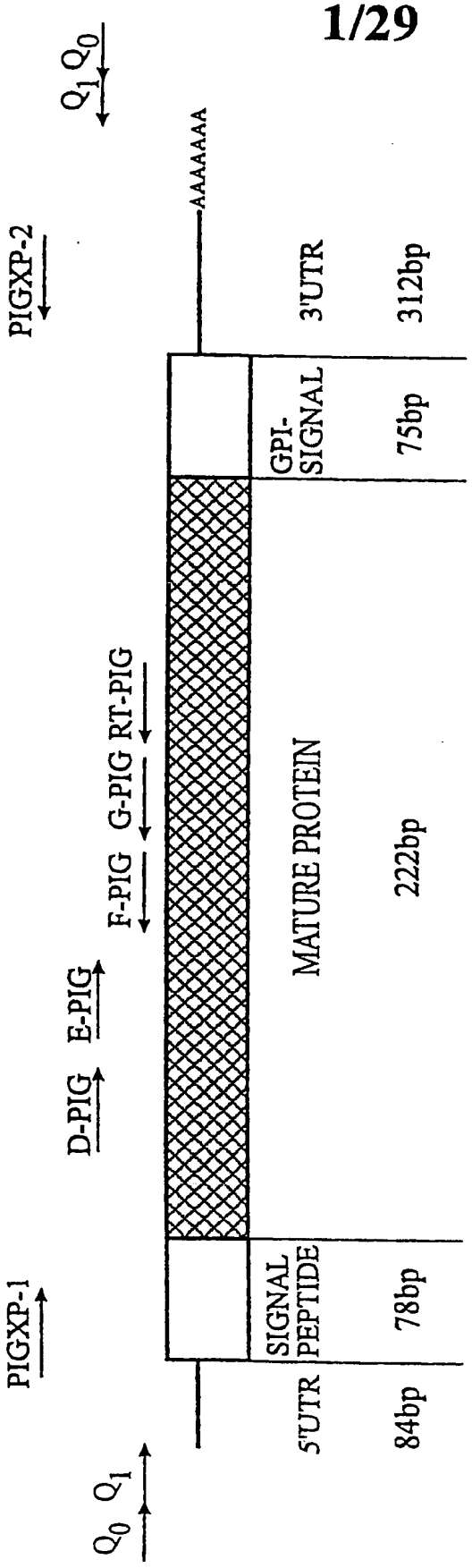

Q1 Q0
AAAAAA
PIGXP-2
3'UTR
312bp
GPI-SIGNAL
75bp
F-PIG G-PIG RT-PIG
MATURE PROTEIN
222bp
D-PIG E-PIG
PIGXP-1
SIGNAL PEPTIDE
78bp
5'UTR
84bp
Q0 Q1

Fig. 1

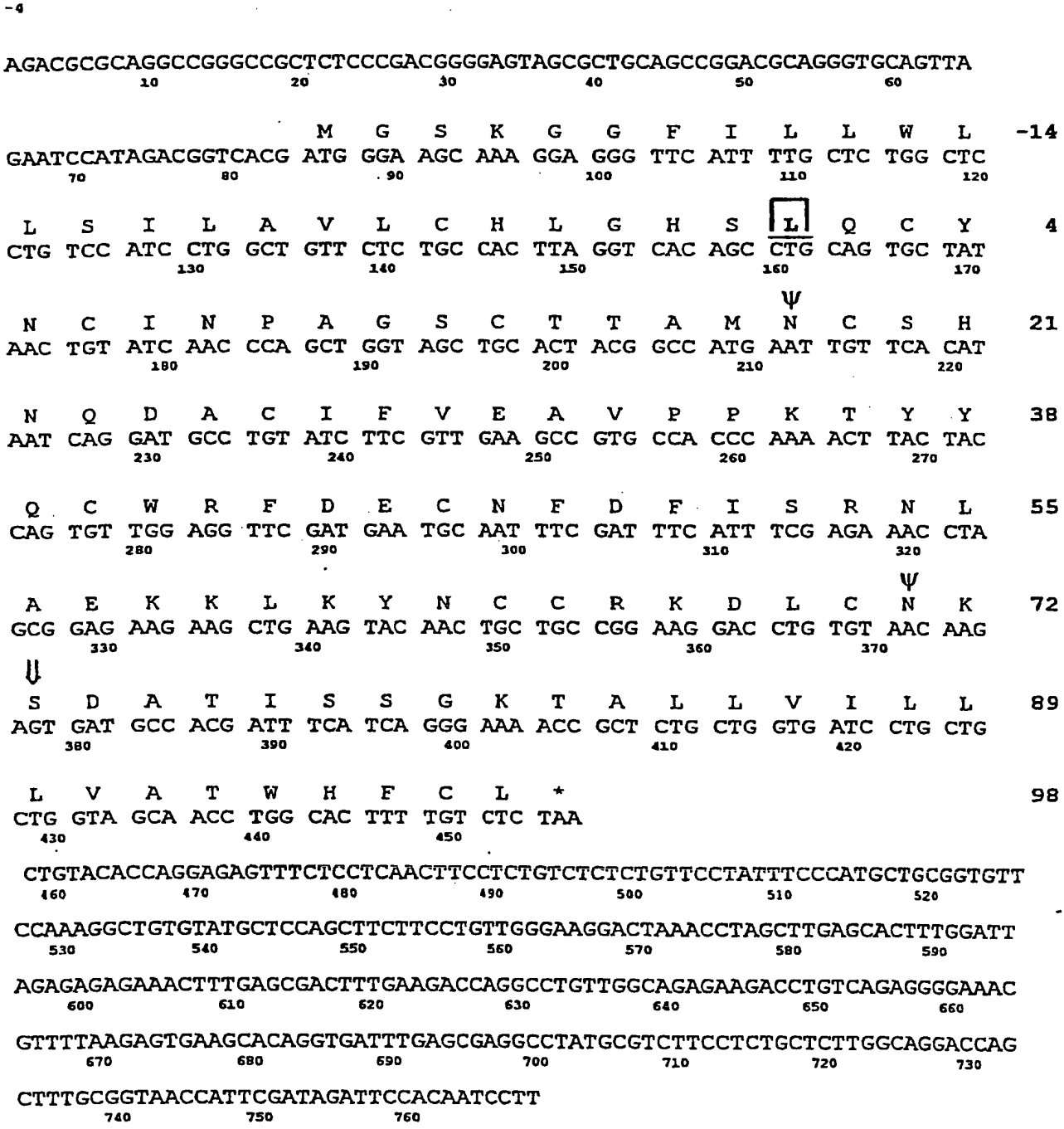


Fig. 2

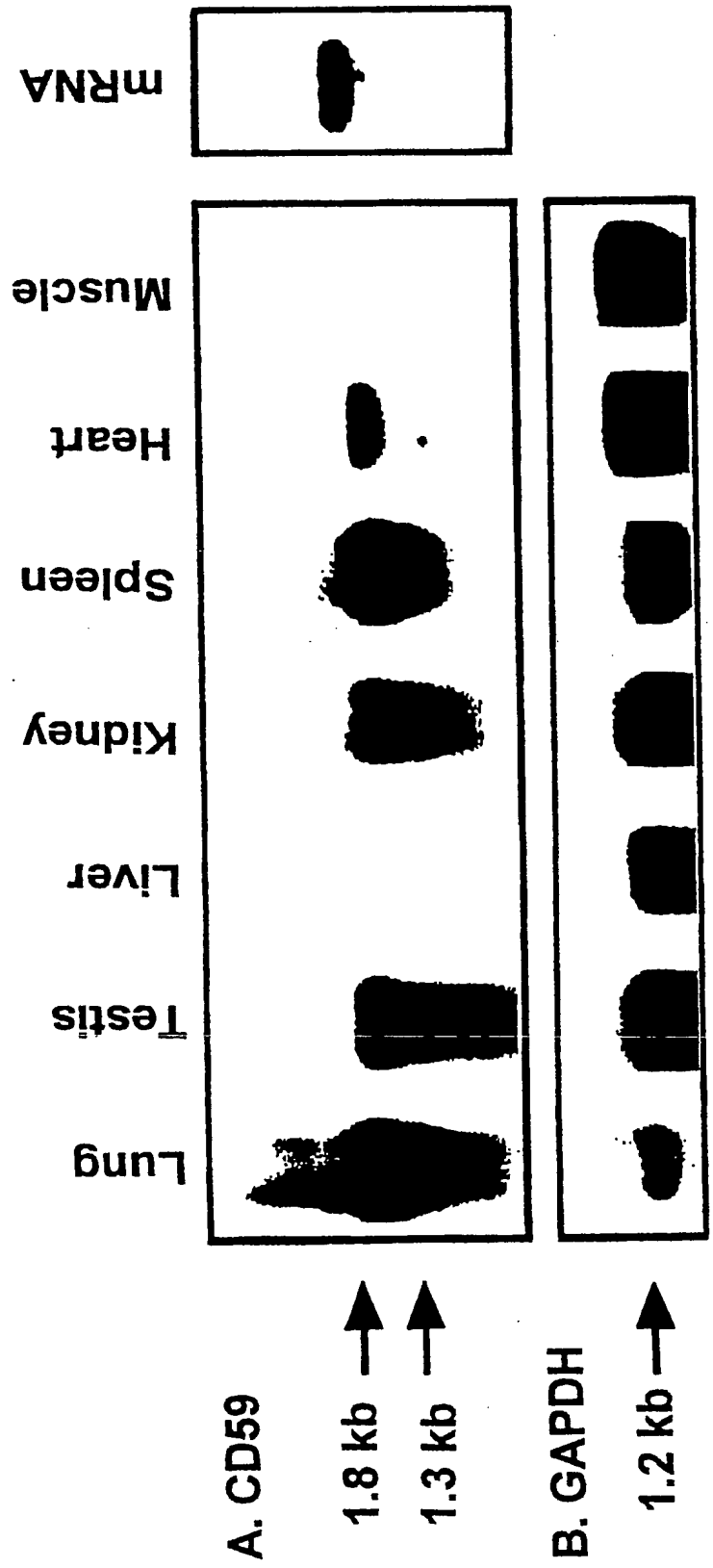

mRNA
Muscle
Heart
Spleen
Kidney
Liver
Testis
Lung
A. CD59
1.8 kb
1.3 kb
B. GAPDH
1.2 kb

Fig. 3

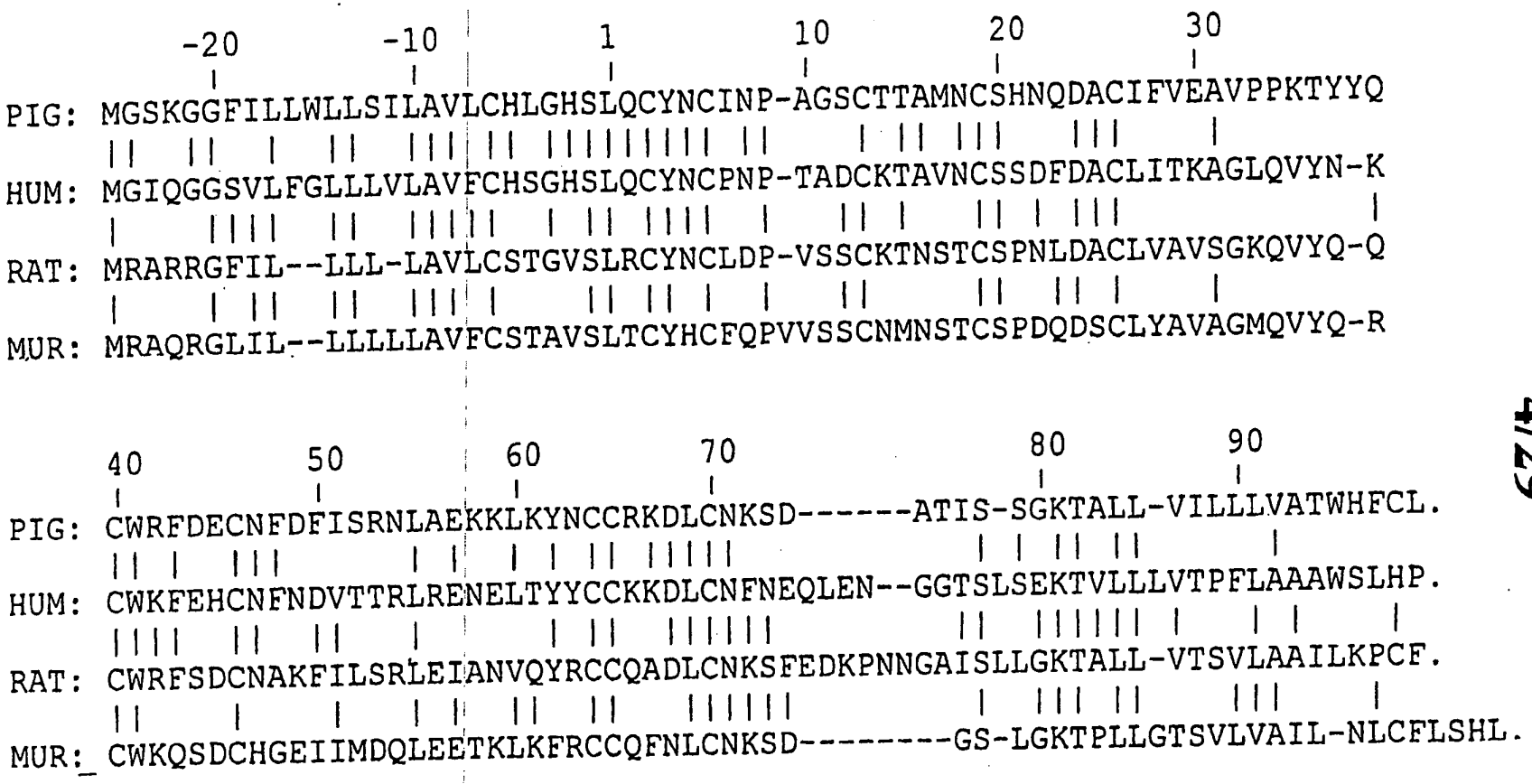

-20 -10 1 10 20 30
PIG: MGSKGGFILLWLLSILAVLCHLGHSLQCYNCINP-AGSCTTAMNCSHNQDACIFVEAVPPKTYYQ
HUM: MGIQGGSVLFGLLLVLAVFCHSGHSLQCYNCPNP-TADCKTAVNCSSDFDACLITKAGLQVYN-K
RAT: MRARRGFIL--LLL-LAVLCSTGVSLRCYNCLDP-VSSCKTNSTCSPNLDACLVAVSGKQVYQ-Q
MUR: MRAQRGLIL--LLLLLAVFCSTAVSLTCYHCFQPVVSSCNMNSTCSPDQDSCLYAVAGMQVYQ-R
40 50 60 70 80 90
PIG: CWRFDECNFDFISRNLAEKKLKYNCCRKDLCNKSD------ATIS-SGKTALL-VILLLVATWHFCL.
HUM: CWKFEHCNFNDVTTRLRENELTYYCCKKDLCNFNEQLEN--GGTSLSEKTVLLLVTPFLAAAWSLHP.
RAT: CWRFSDCNAKFILSRLEIANVQYRCCQADLCNKSFEDKPNNGAISLLGKTALL-VTSVLAAILKPCF.
MUR: CWKQSDCHGEIIMDQLEETKLKFRCCQFNLCNKSD--------GS-LGKTPLLGTSVLVAIL-NLCFLSHL.

Fig. 4

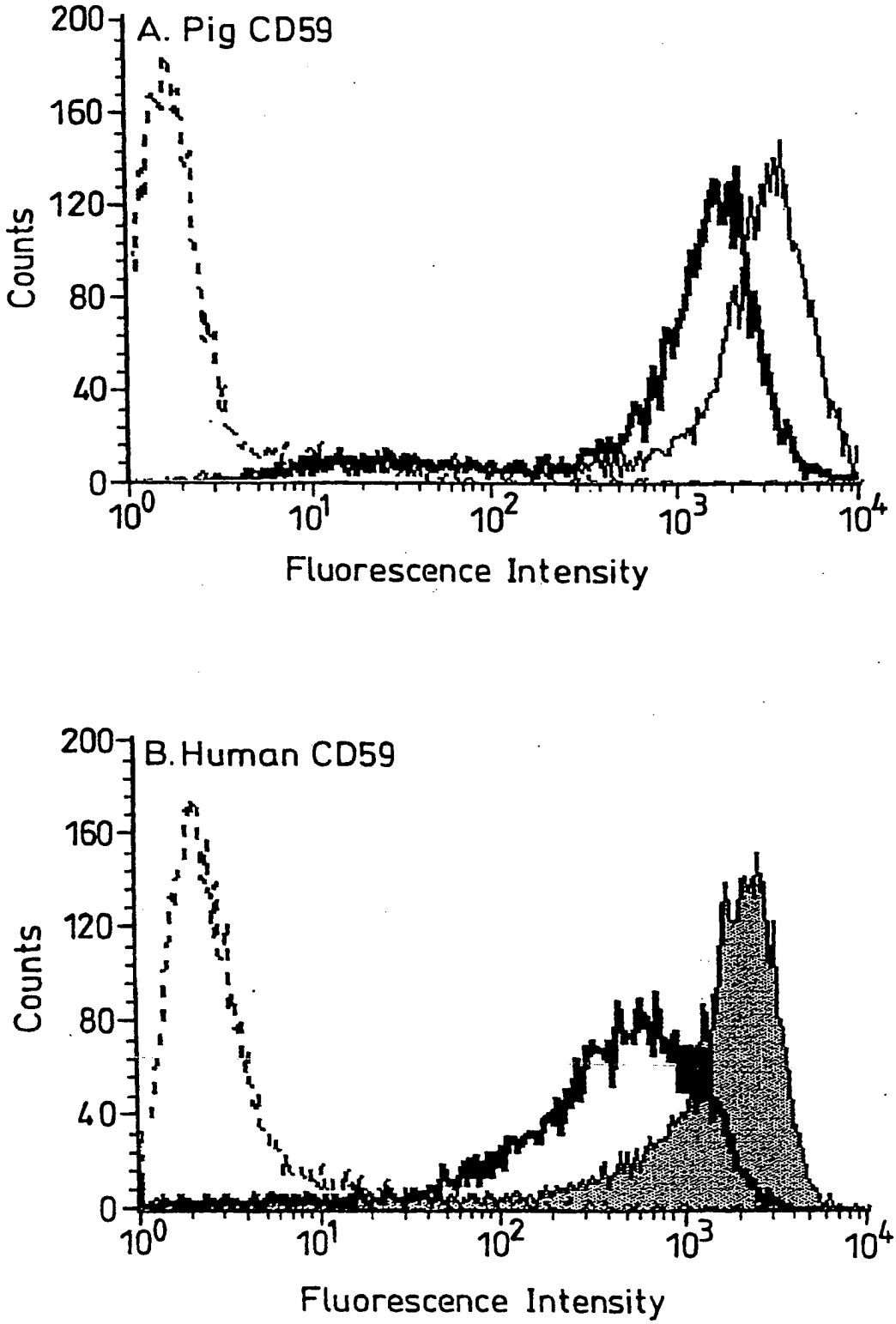

A. Pig CD59
Counts
200
160
120
80
40
0
10^0
10^1
10^2
10^3
10^4
Fluorescence Intensity
B. Human CD59
Counts
200
160
120
80
40
0
10^0
10^1
10^2
10^3
10^4
Fluorescence Intensity

Fig. 5

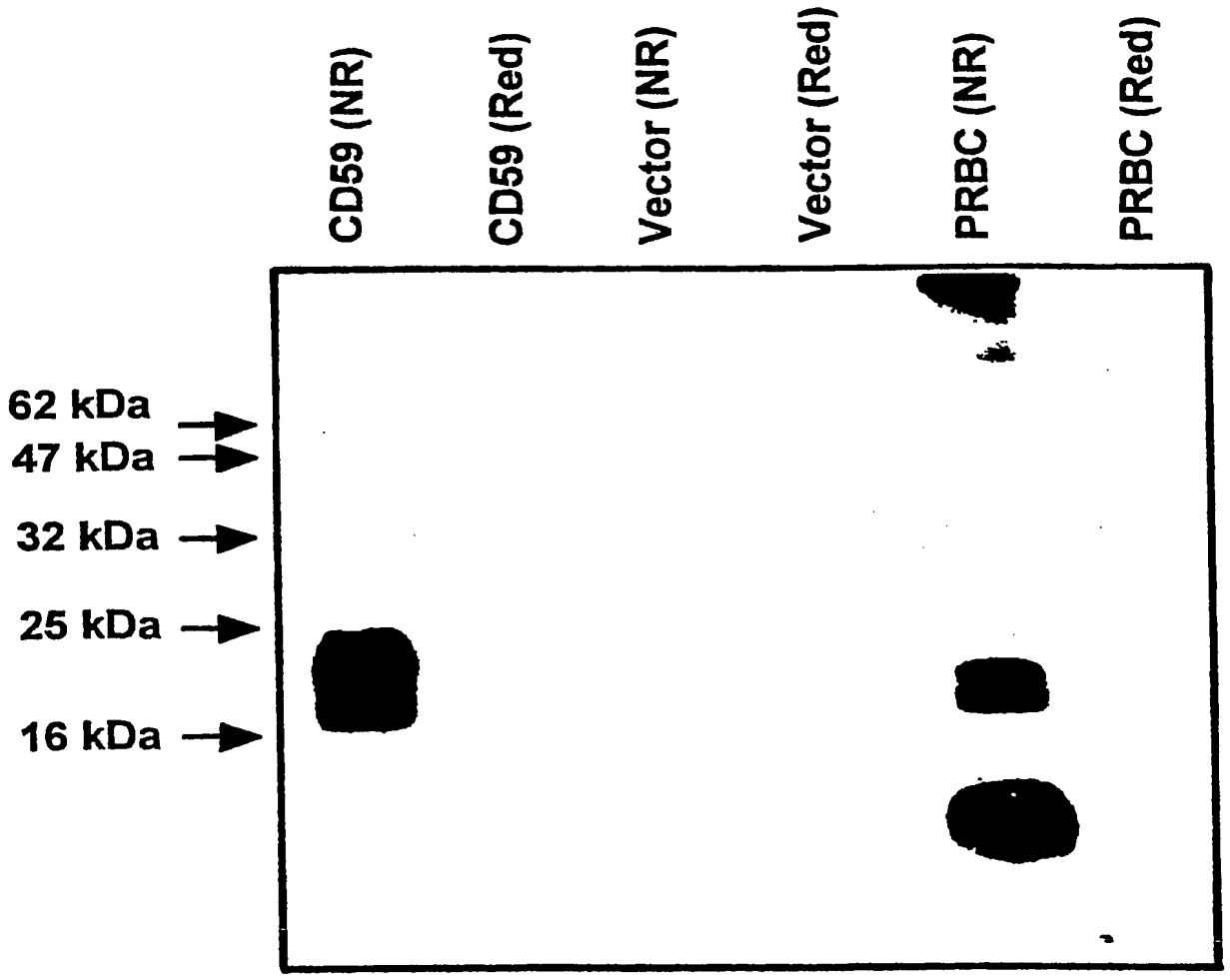
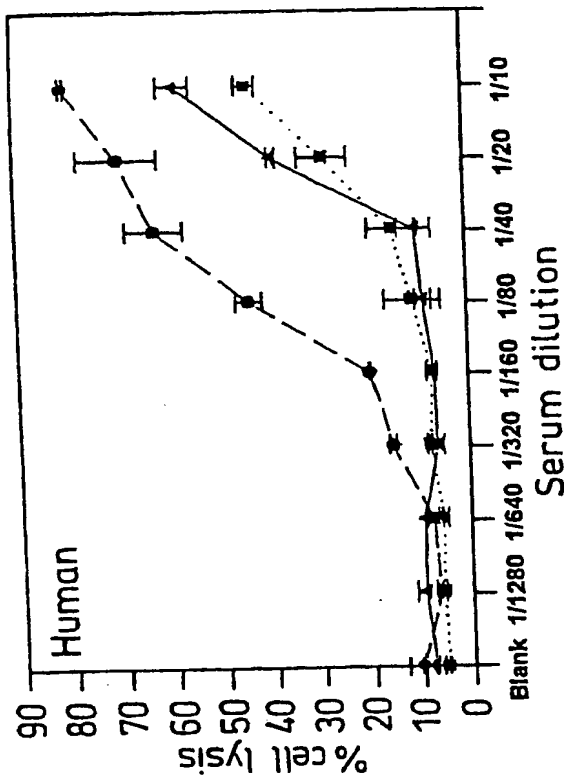
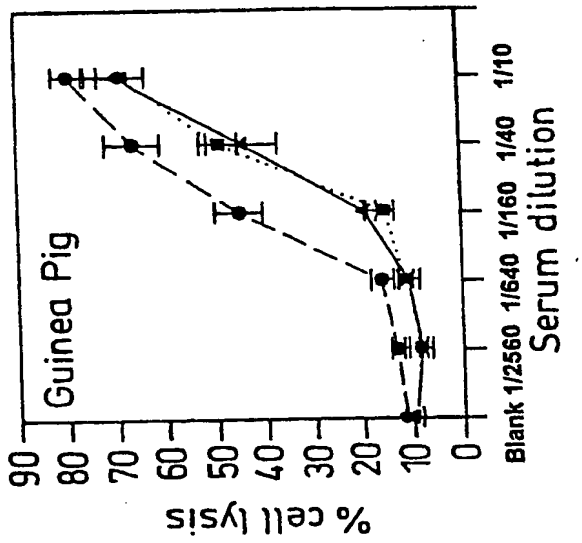
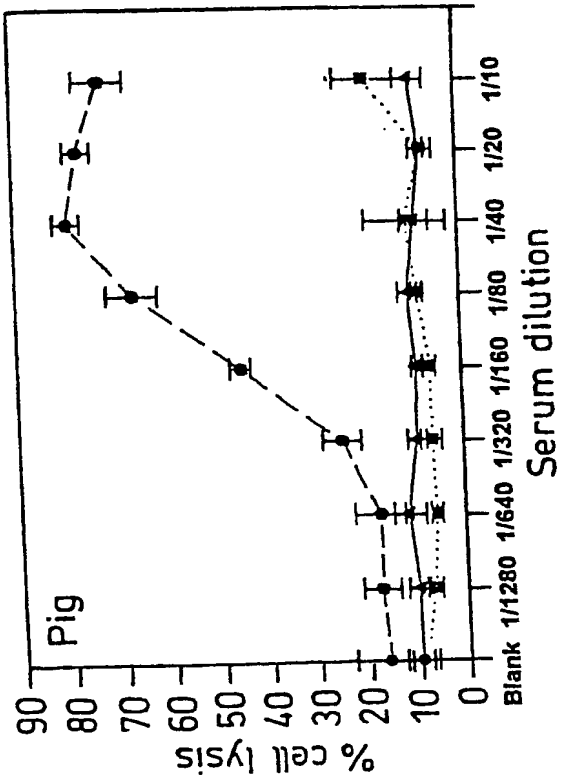

CD59 (NR)
CD59 (Red)
Vector (NR)
Vector (Red)
PRBC (NR)
PRBC (Red)
62 kDa
47 kDa
32 kDa
25 kDa
16 kDa

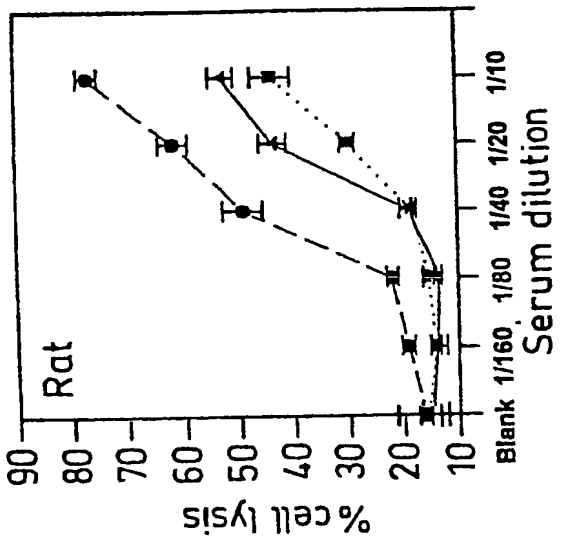
Fig. 6

Fig. 7 (part 1 of 2)









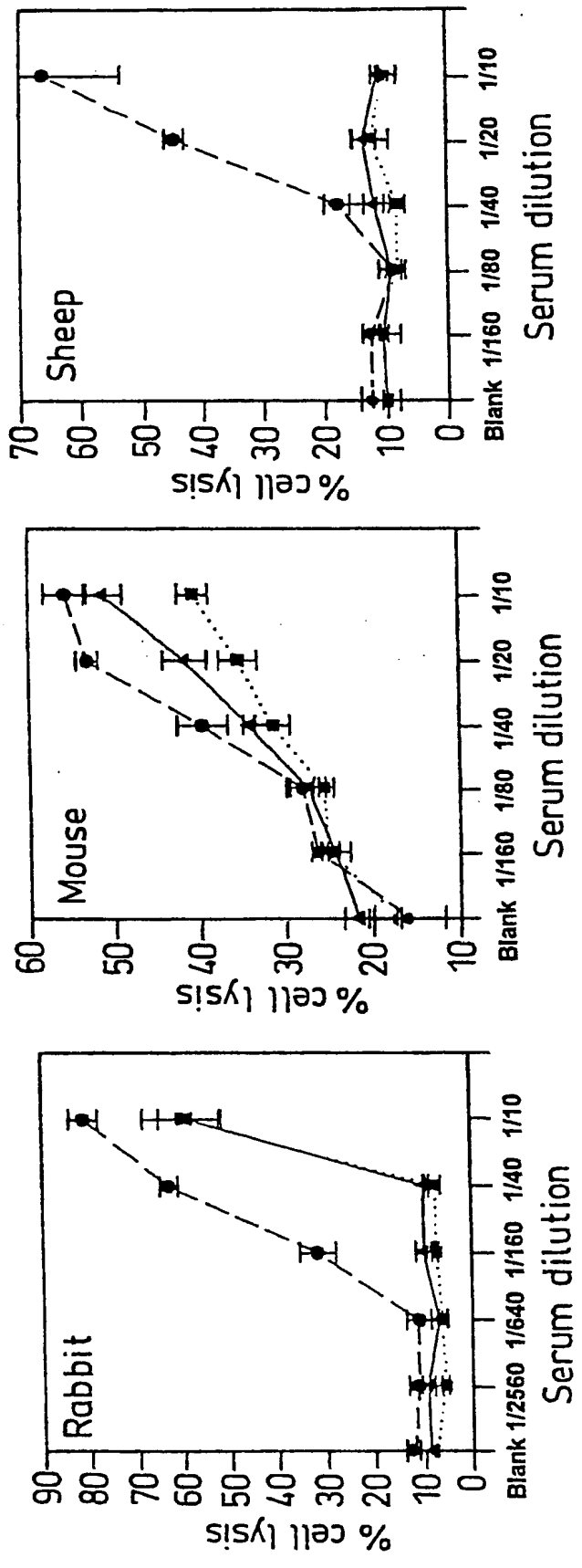


Fig. 7 (part 2 of 2)

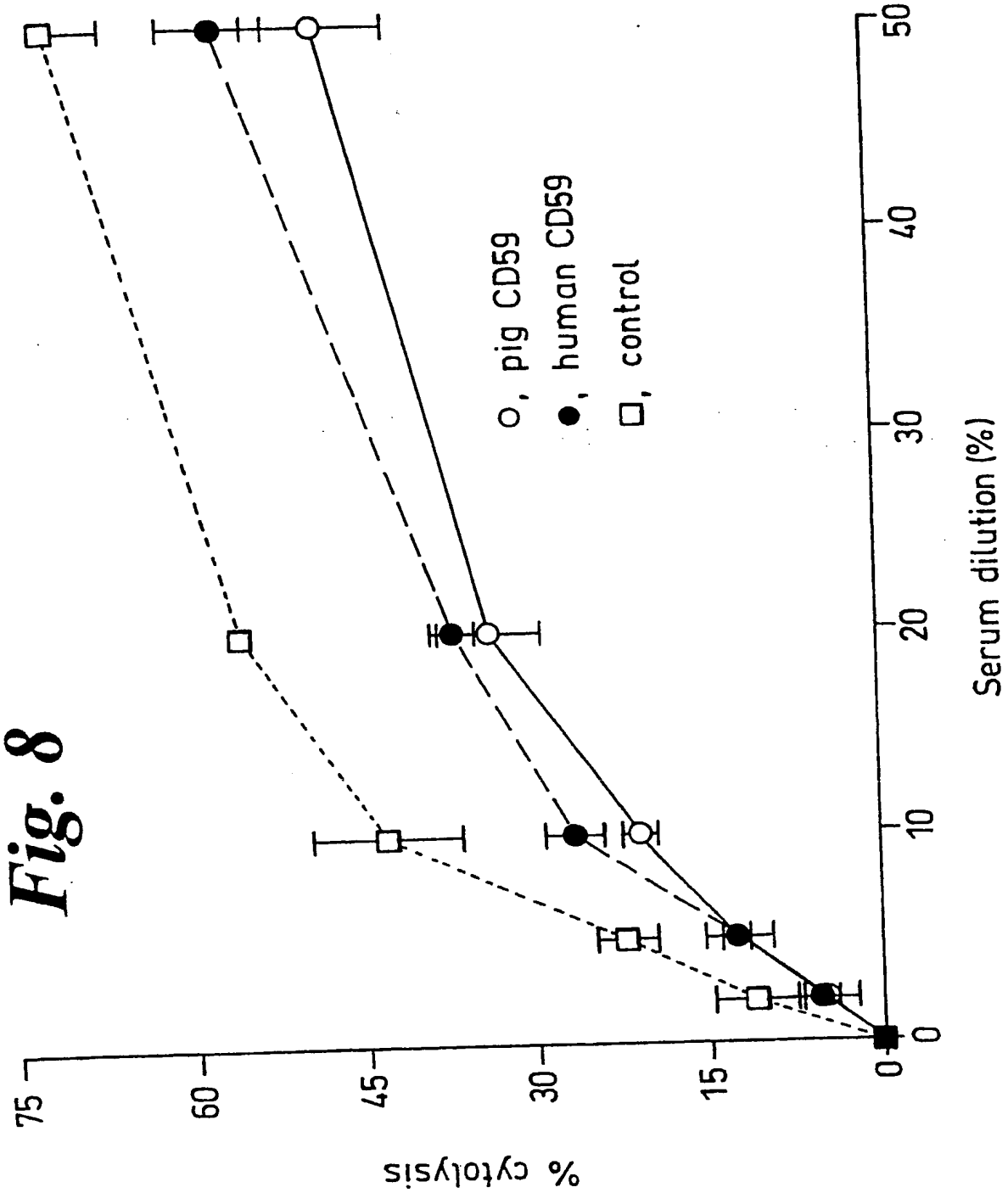
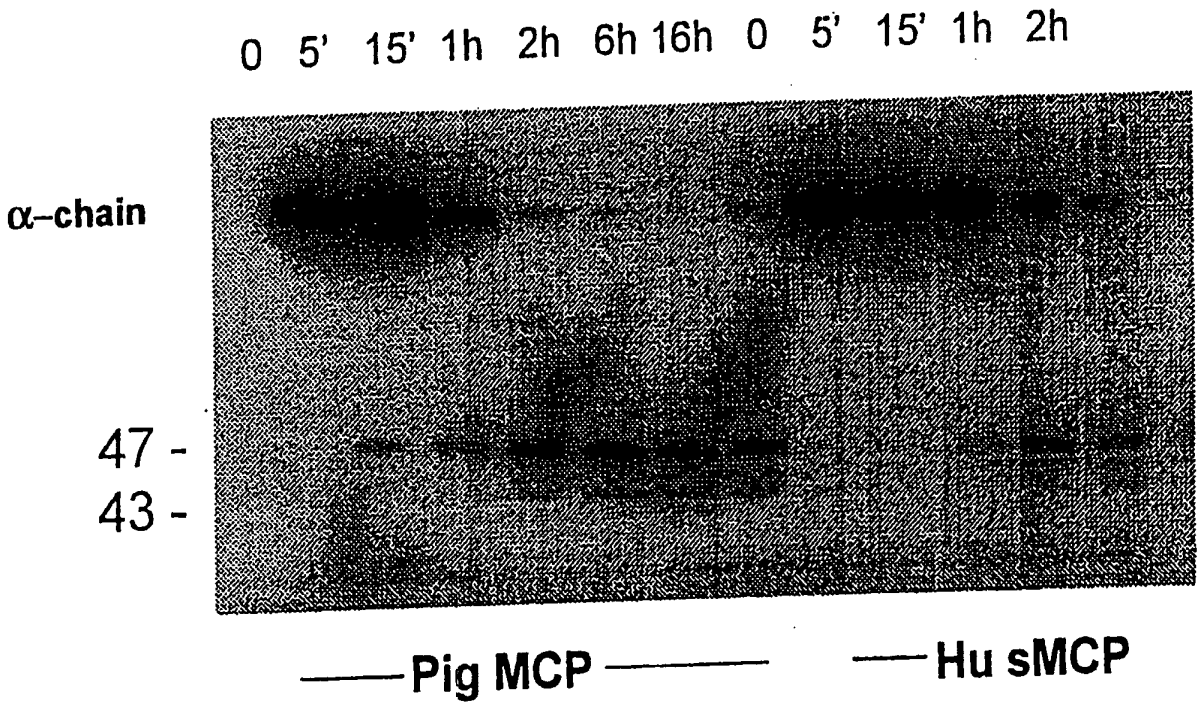

% cytolysis
75
60
45
30
15
0
0
10
20
30
40
50
Serum dilution (%)
○, pig CD59
●, human CD59
□, control

Fig. 8

Time course Cofactor activity: pig MCP vs Hu sMCP

Fig. 9



500 ng C3 was incubated with 50 ng factor I and 50 ng pig MCP or human sMCP

Pig MCP is a better cofactor than Hu sMCP for human C3 and human factor I

Dose/response Cofactor activity: pig MCP vs Hu sMCP

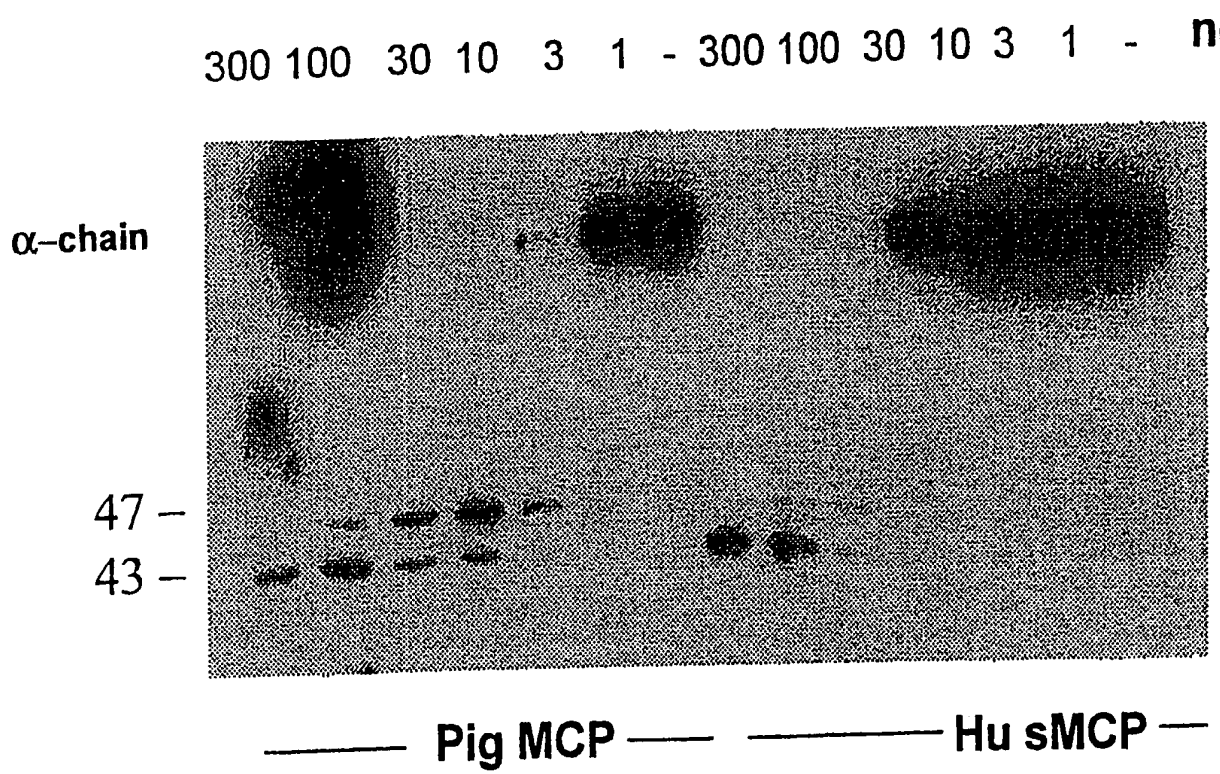


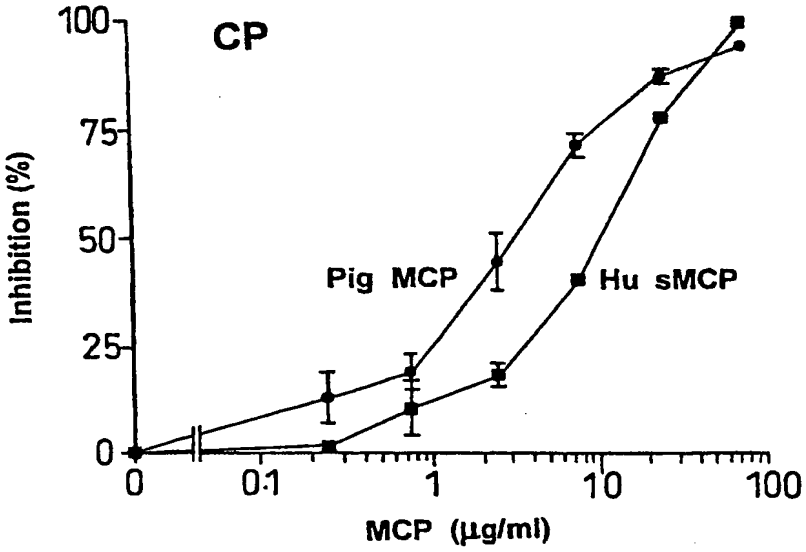
Fig. 10

500 ng C3 was incubated with 50 ng factor I and various amounts of pig MCP or human sMCP for 16 at 37°C. W.blot of reduced samples, probed with anti Hu C3c

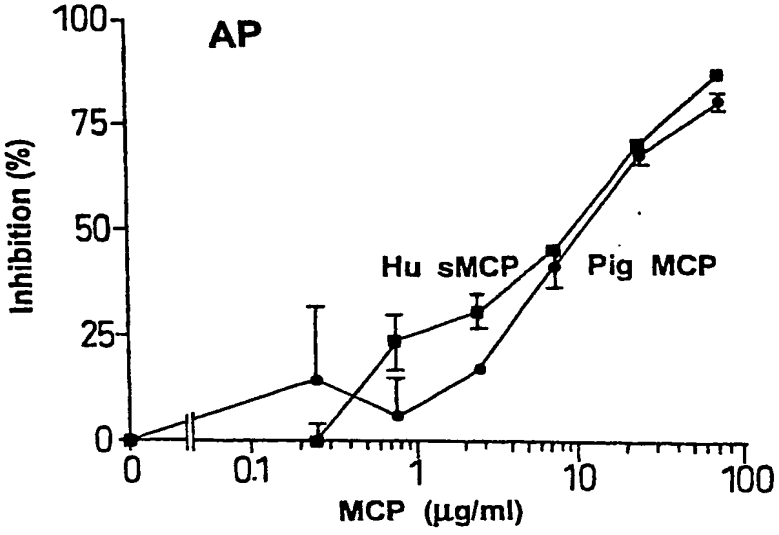
Pig MCP is a better cofactor than Hu sMCP for human C3 and human factor I

Fig. 11

Inhibition of CP and AP of human serum by human sMCP and pig MCP



RaE were incubated with human serum in the presence of Hu soluble MCP or pig MCP under CP or AP conditions.



Pig MCP is a better regulator of the CP of human C than human sMCP.
Pig MCP and Hu sMCP have similar activity in regulation of the human AP.

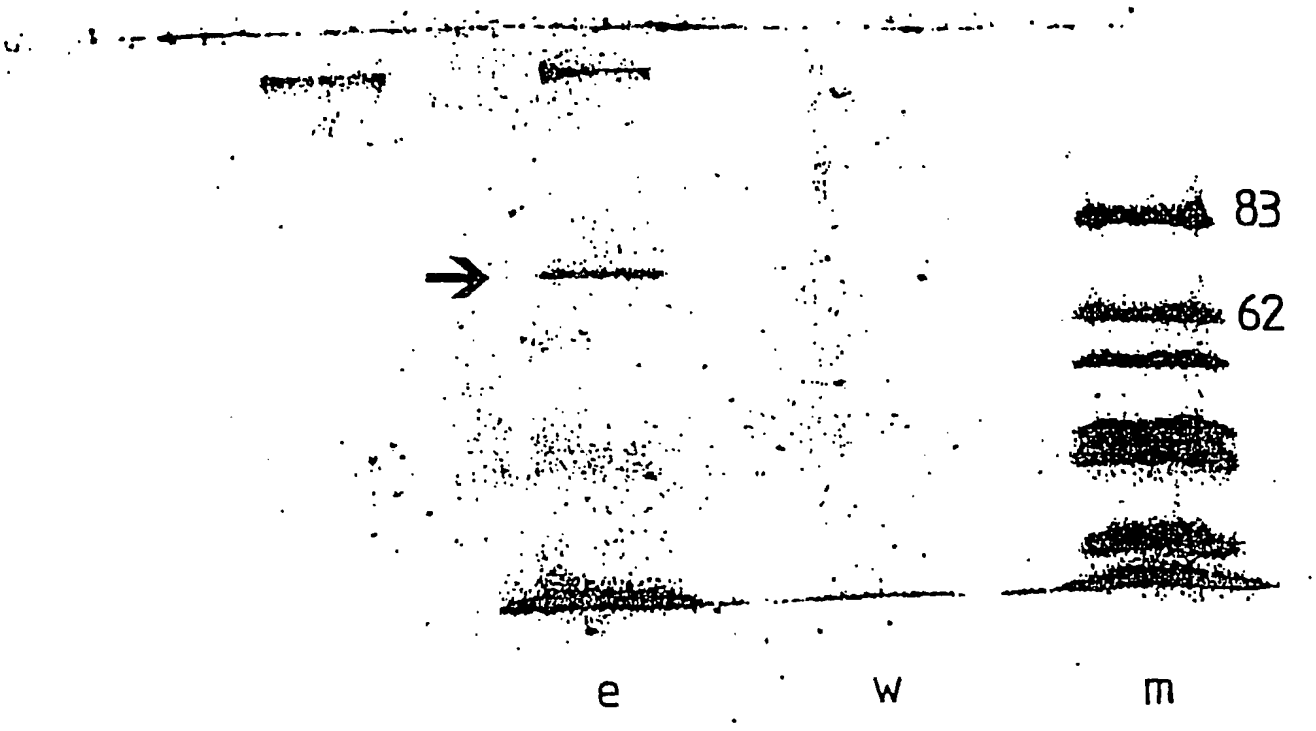


Fig. 12

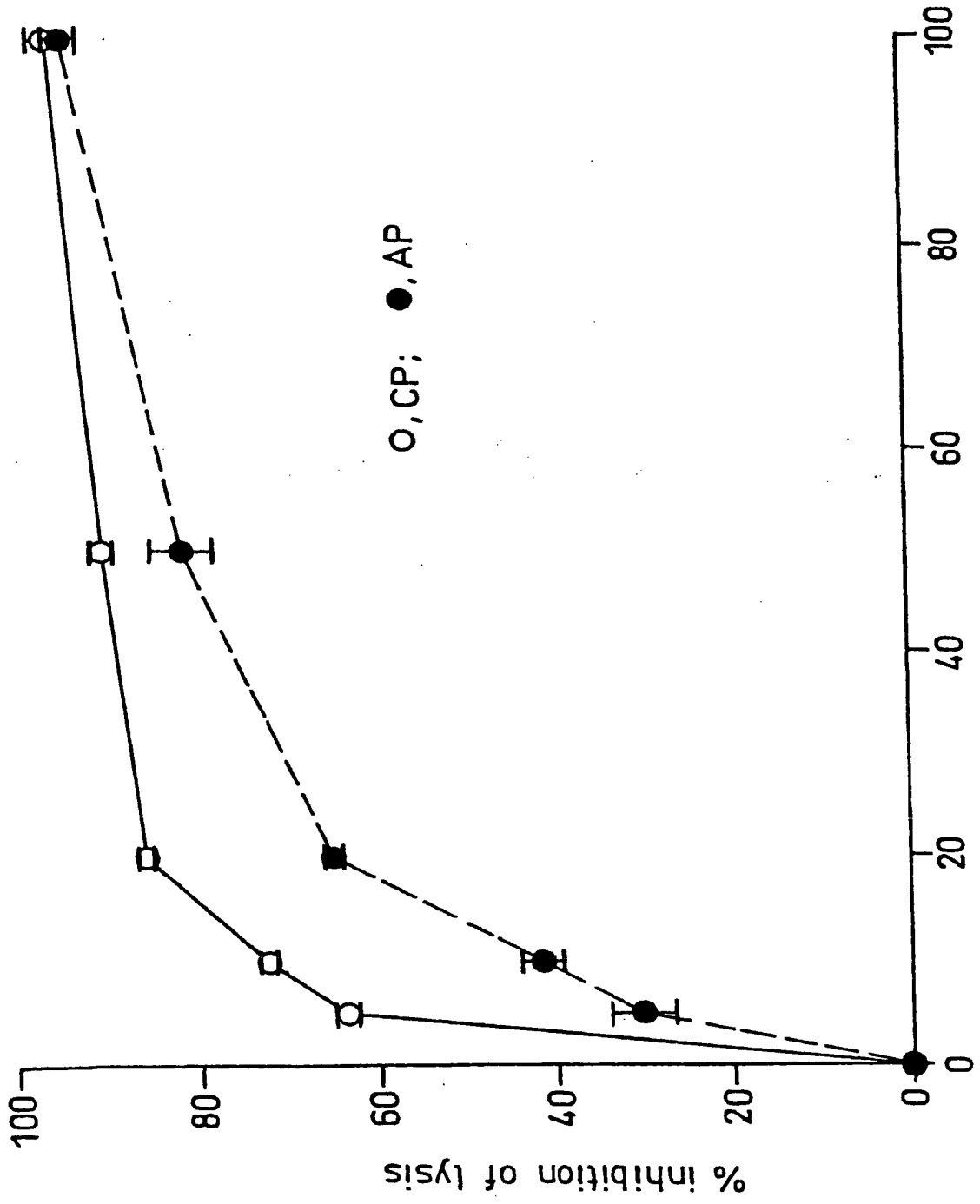

% inhibition of lysis
0
20
40
60
80
100
O,CP; ●,AP
DAF added (ug/ml)
0
20
40
60
80
100

Fig. 13

pDAF-7 cDNA sequence:

CCACCGCGGTGGCGGCNCGCTCTAGAACTAGTGGATCCCCCGGGCTGCAG
GAATTCGGCACGAGATTTCGTCTTAATCGCGGAGGTCGCAGAGTCCGGGA
GCCGCTCGGGGTCCCCGTTCCCGCGCGCCATGAGTCCCCTGCCGCGGAGC
GCCCCCGCGGTGAGGCGCCTAATGGGCGGACAGACGCCGCCGCCGCTGCT
GCTGCTGCTGCTGCTGCTGTGTATCCCGGCTGCGCAGGGTGACTGCAGCC
TTCCACCCGATGTACCTAATGCCCAACCAGATTTGCGAGGTCTTGCAAGT
TTTCCTGAACAAACCACAATAACATACAAATGTAACAAAGGCTTTGTCAA
AGTTCCTGGCATGGCAGACTCAGTGCTCTGTCTTAATGATAAATGGTCAG
AAGTTGCAGAATTTTGTAATCGTAGCTGTGATGTTCCAACCAGGCTACAT
TTTGCATCTCTTAAAAAGTCTTACAGCAAACAGAATTATTTCCCAGAGGG
TTTCACCGTGGAATATGAGTGCCGTAAGGGCTATAAAAGGGATCTTACTC
TATCAGAAAAACTAACTTGCCTTCAGAATTTTACGTGGTCCAAACCTGAT
GAATTTTGCAAAAAAAAACAATGTCCGACTCCTGGAGAACTAAAAAATGG
TCATGTCAATATAACAACTGACTTGTTATTTGGCGCATCCATCTTTTTCT
CATGTAACGCAGGGTACAGACTAGTTGGTGCAACTTCTAGTTACTGtTTT
GCCATAGCAAATGATGTTGAGTGGAGTGATCCATTGCCAGATTGCCAAGA
AATTTCTCCAACTGTCAAAGCCATACCAGCTGTTGAGAAACCCATCACAG
TAAATTTTCCAGCAACAAAGTATCCAGCTATTCCCAGGGCCACAACGAGT
TTTCATTCAAGTACATCTAAAAATCGAGGAAACCCTTCTTCAGGCATGAG
AATCATGTCGTCTGGTACCATGCTACTTATTGCAGGAGGTGTTGCTGTTA
TTATAATAATTGTTGCCCTAATTCTAGCCAAAGGTTTCTGGCACTATGGA
AAATCAGGCTCTTACCACACTCATGAGAACAACAAAGCCGTTAATGTTGC
ATTTTATAATTTACCTGCGACTGGCGATGCCGCAgATGTAAGACCTGGTA
ATTAACAAAAGGACGTGCATGTGTAACACTGACAGTTTTGCTTATGGTGC
TAGTAACCATTGGCTAGCTGACTTAGCCAAAGAAGAGTTAAGAAGAAAGT
GCACACAAGTACACAGAATATTTTCAGTTTCTTAAAACTTTCAGGTGGGA
GTGGACATAGTTTGTGGTAGTGNTCTTCGNTTTGCATGGTTTCATTGGCT
CTAAGGNACATAGGAATGCACAGAACCNAAGAGAAACAAATCTATCCTGA
AANTACATCCTCAACACTTCTAANACTCTTGGAAATNGAACAAGNTCATA
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GGTCATGGGTAGNAATTTTNGAAAAANGAAACCCNAAAGGGGANTTTTCC
CCCCCAAAGGGGNAAGGGTATTTTTATTTAATTAAGGNAAAAAAAAAAAA
AAAAACCCNNNGGGGGGGCCCGGGNCCCATTTTCCCT

pDAF-14 cDNA sequence:

CACGAGCCGCCGCCGCTGCTGCTGCTGCTGCTGCTGCTGTGTATCCCGGC
TGCGCAGGGTGACTGCAGCCTTCCACCCGATGTACCTAATGCCCAACCAG
ATTTGCGAGGTCTTGCAAGTTTTCCTGAACAAACCACAATAACATACAAA
TGTAACAAAGGCTTTGTCAAAGTTCCTGGCATGGCAGACTCAGTGCTCTG
TCTTAATGATAAATGGTCAGAAGTTGCAGAATTTTGTAATCGTAGCTGTG
ATGTTCCAACCAGGCTACATTTTGCATCTCTTAAAAAGTCTTACAGCAAA
CAGAATTATTTCCCAGAGGGTTTCACCGTGGAATATGAGTGCCGTAAGGG
CTATAAAAGGGATCTTACTCTATCAGAAAAACTAACTTGCCTTCAGAATT
TTACGTGGTCCAAACCTGATGAATTTTGCAAAAAAAAAACAATGTCCGACT
CCTGGAGAACTAAAAAATGGTCATGTCAATATAACAACTGACTTGTTATT
TGGCGCATCCATCTTTTTCTCATGTAACGCAGGGTACAGACTAGTTGGTG
CAACTTCTAGTTACTGtTTTGCCATAGCAAATGATGTTGAGTGGAGTGAT
CCATTGCCAGAaTGCCAAGAAATTTCTCCAACTGTCAAAGCCaTACCAGC
TGTTGAGAAACCCATCACAGTAAATTTTCCAGGTACCAAAGCCCTATCAT
CTCCTCAGAAACCCTCCACAGCAAATACTCTAGCTACAGAGTTACTACCA
ACTCCTCAGGAACCCACCACAGTAAATGTTCCAGATAGTAAAGCCATATC
ATCTCCTCAGAAACCCTCCACAGTAAATACTCCAGCTACAGACTTACTAC
CAACTCCTCAGGAACCCACCACAGTAAAtGTTCCAGATAGTAAAGCCATA
TCATCTTCTCAGAAACCCTCCACAGTAAATACTCCAGCTCAGACTTACTA
CCAACTCCTCAGGAACCCACCACAGTaA

Fig. 14

pDAF-7, predicted protein sequence:

```
MGGQTPPPLLLLLLLLLCIPAAQGDCSLPPDVPNAQPDLRGLASFPEQTTI
TYKCNKGFVKVPGMADSVLCLNDKWSEVAEFCNRSCDVPTRLHFASLKKS
YSKQNYFPEGFTVEYECRKGYKRDLTLSEKLTCLQNFTWSKPDEFCKKKQ
CPTPGELKNGHVNITTDLLFGASIFFSCNAGYRLVGATSSYCFAIANDVE
WSDPLPDCQEISPTVKAIPAVEKPITVNFPATKYPAIPRATTSFHSSTSK
NRGNPSSGMRIMSSGTMLLIAGGVAVIIIIVALILAKGFWHYGKSGSYHT
HENNKAVNVAFYNLPATGDAADVRPGN.
```

pDAF-14, predicted protein sequence:

```
   HEPPPLLLLLLLLLCIPAAQGDCSLPPDVPNAQPDLRGLASFPEQTTI
TYKCNKGFVKVPGMADSVLCLNDKWSEVAEFCNRSCDVPTRLHFASLKKS
YSKQNYFPEGFTVEYECRKGYKRDLTLSEKLTCLQNFTWSKPDEFCKKKQ
CPTPGELKNGHVNITTDLLFGASIFFSCNAGYRLVGATSSYCFAIANDVE
WSDPLPECQEISPTVKAIPAVEKPITVNFPGTKALSSPQKPSTANTLATE
LLPTPQEPTTVNVPDSKAISSPQKPSTVNTPATDLLPTPQEPTTVNVPDS
KAISSSQKPSTVNTPAQTYYQLLRNPPQ.
```

Alignment with human DAF (conserved residues marked as *):

```
1        10        20        30        40        50
 PSVPAALPLLGELPRLLLLVLLCLPAVWGDCGLPPDVPNAQPALEGRTS     HuDAF
              • ••••  ••• ••  ••• ••••••••• • •  •
MGGQTPP-------PLLLLLLLLLCIPAAQGDCSLPPDVPNAQPDLRGLAS     pDAF-7

51       60        70        80        90       100
FPEDTVITYKCEESFVKIPGEKDSVTCLKGMQWSDIEEFCNRSCEVPTRL     HuDAF
••• • •••••   ••• ••  ••• ••    ••   •••••• •••••
FPEQTTITYKCNKGFVKVPGMADSVLCLND-KWSEVAEFCNRSCDVPTRL     pDAF-7

101      110       120       130       140      150
NSASLKQPYITQNYFPVGTVVEYECRPGYRREPSLSPKLTCLQNLKWSTA     HuDAF
 ••••  • ••••• •  •••••• •• •  •• ••••••• ••
HFASLKKSYSKQNYFPEGFTVEYECRKGYKRDLTLSEKLTCLQNFTWSKP     pDAF-7

151      160       170       180       190      200
VEFCKKKSCPNPGEIRNGQIDVPGGILFGATISFSCNTGYKLFGSTSSFC     HuDAF
 •••••• •• •••  ••        •••• • •••• •• • • ••• •
DEFCKKKQCPTPGELKNGHVNITTDLLFGASIFFSCNAGYRLVGATSSYC     pDAF-7

201      210       220       230       240      250
LISGSSVQWSDPLPECREIYCPAPPQIDNGIIQGERDHYGYRQSVTYACN     HuDAF
      • •••••• • ••
FAIANDVEWSDPLPDCQEI-------------------------------     pDAF-7
                  ↑end SCR3
251
KGFTMIGEHSIYCTVNNDEGEWSGPPPECRGKSLTSKVPPTVQKPTTVNV     HuDAF
                                 • • •  • • •• •••
---------------------------------SPTVKAIPAVEKPITVNF     pDAF-7
                               ↑end SCR4
301
PTTEVSPTSQKTTTKTTTPNAQATRSTPVSRTTKHFHETTPNKGSGTTSG     HuDAF
• •        ••                •            •
PATKYPAIPRATTSFHSSTSKNRGNPSSGMRIMSSGTMLLIAGGVAVIII     pDAF-7
 ↑end STP-A
351
TTRLLSGHTCFTLTGLLGTLVTMGLLT                            HuDAF
 •
IVALILAKGFWHYGKSGSYHTHENNKAVNVAFYNLPATGDAADVRPGN.      pDAF-7
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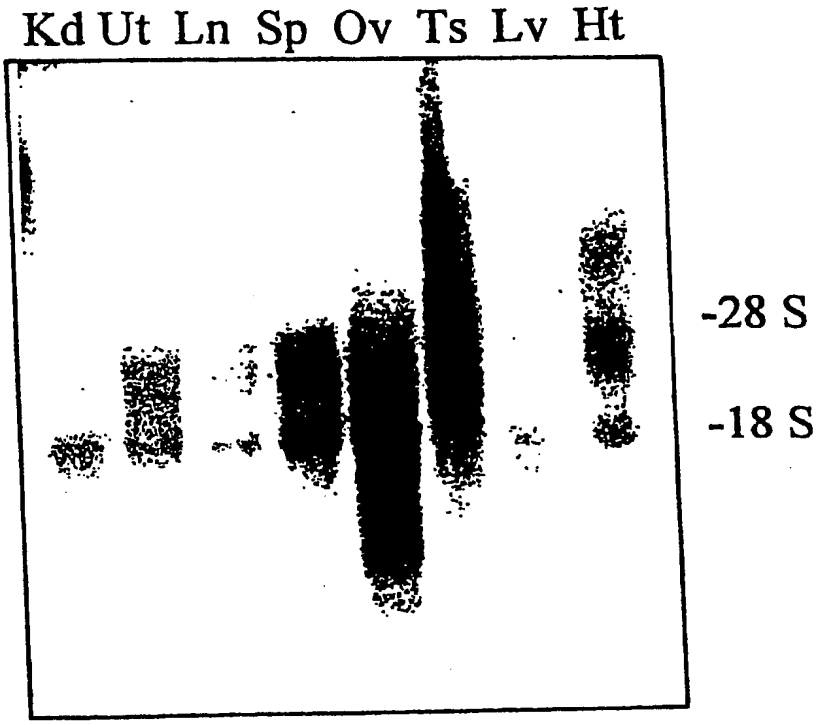
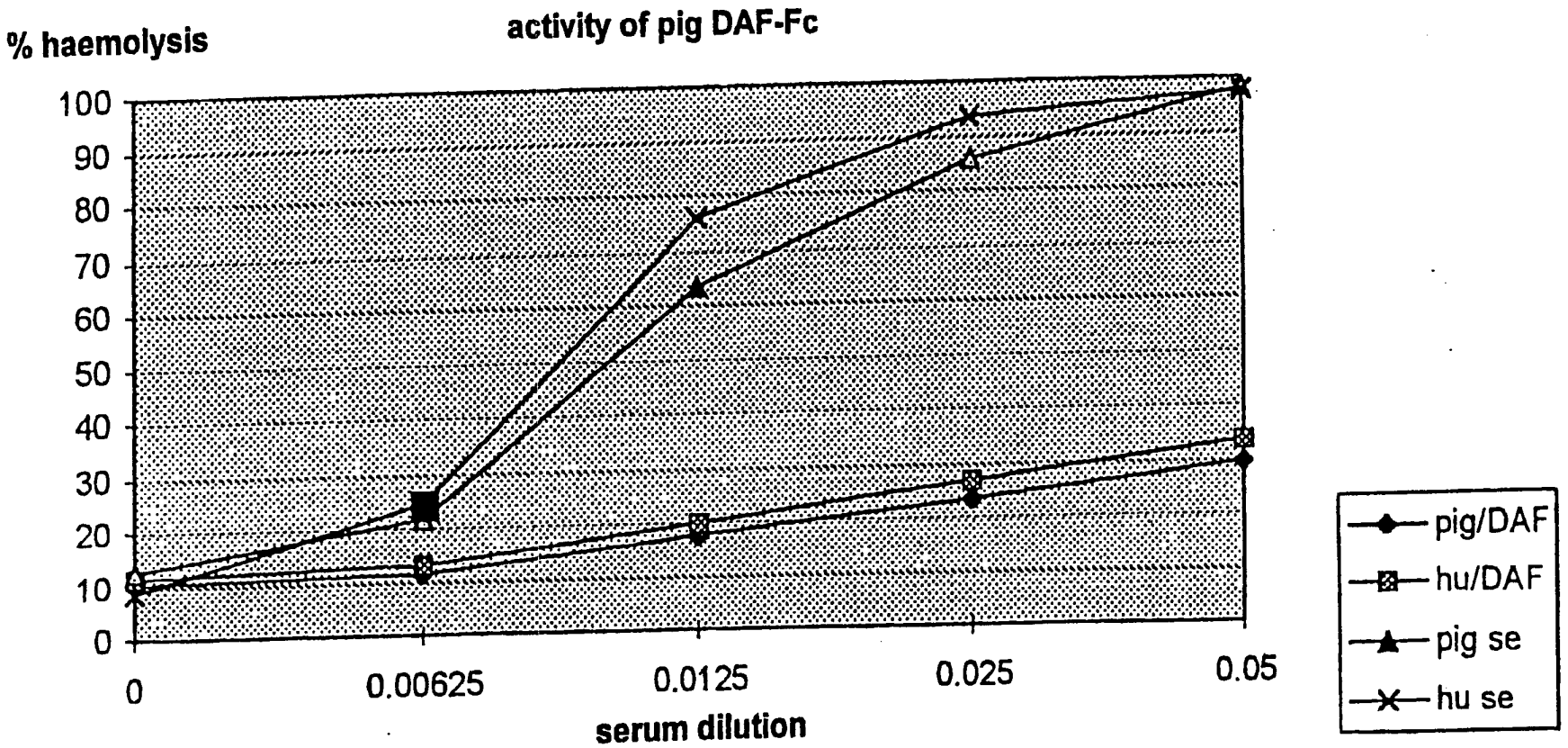
Fig. 15



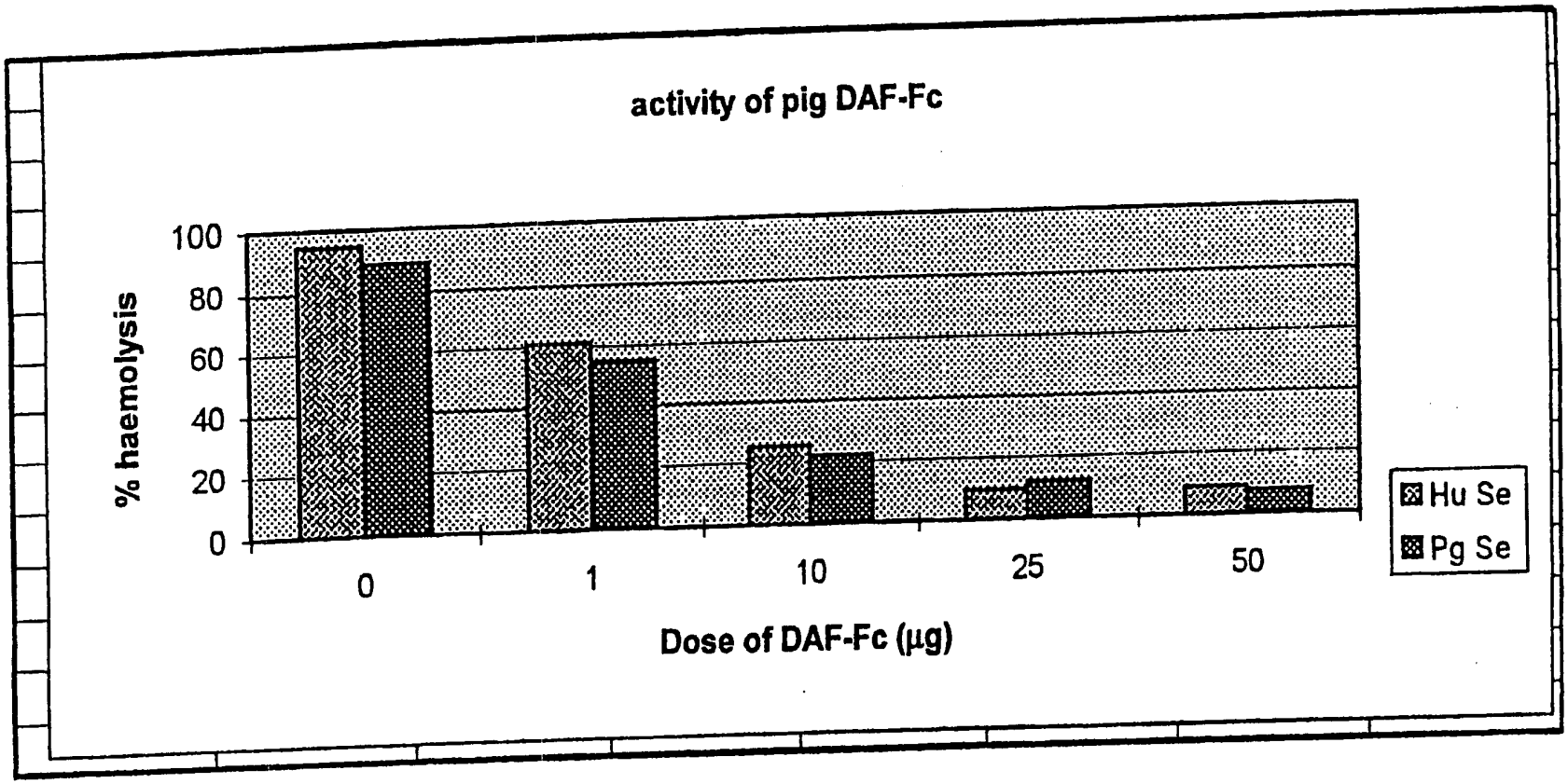
Northern analysis of porcine DAF

Fig. 16



Antibody-sensitised human erythrocytes in GVB were incubated for 30 min at 37°C with various dilutions of pig or human serum in the presence or absence of pig DAF-Fc at 10µg/ml (final). Haemolysis was measured by quantifying haemoglobin release into supernatant.

Fig. 17a **Activity of pig DAF-Fc**



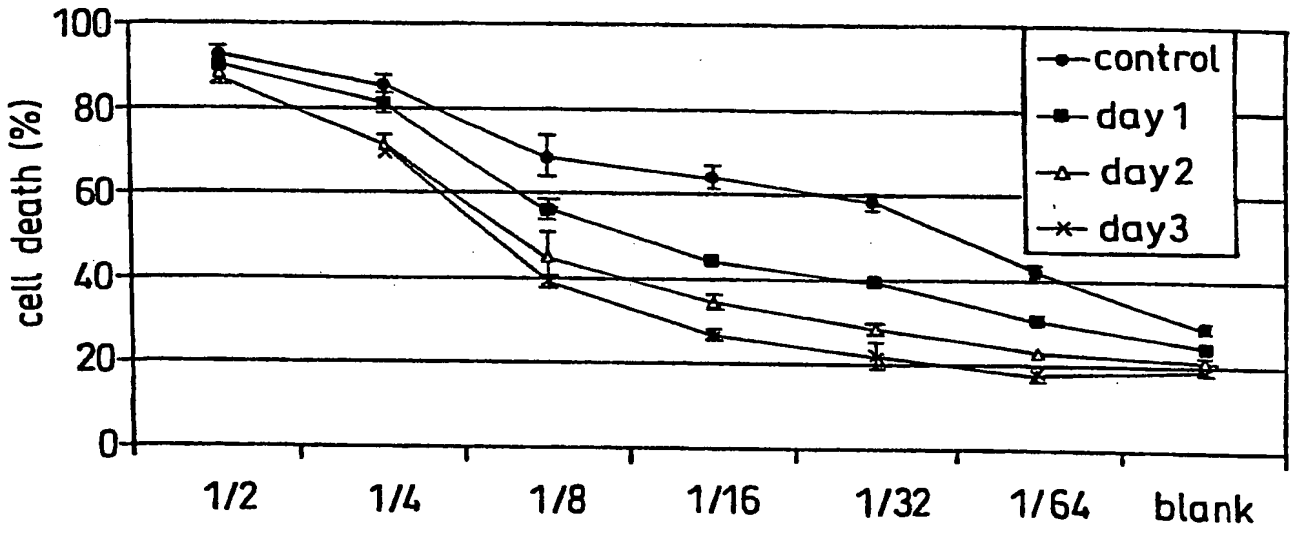
Antibody-sensitised human erythrocytes in GVB were incubated for 30 min at 37°C with a constant dilution of human or pig serum (1:20) and various amounts of pig DAF-Fc (0 - 50µg/ml (final). Haemolysis was measured by quantifying haemoglobin release into supernatant.

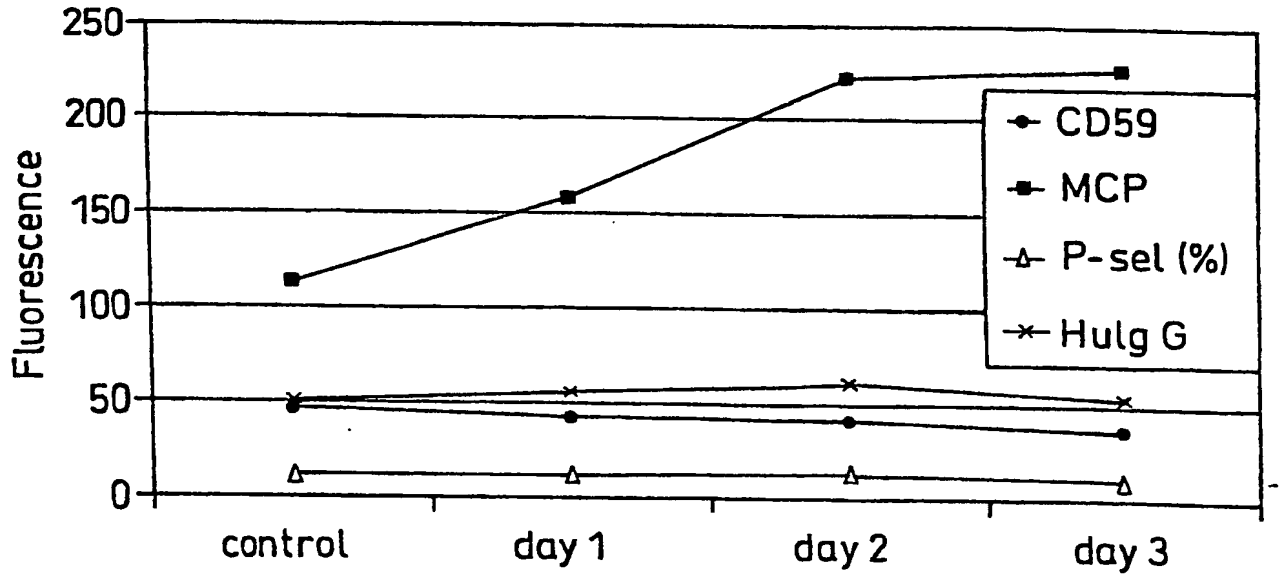
Fig. 17b **Activity of pig DAF-Fc - dose response with human and pig serum**

Effect of PMA on expression of CD59 and MCP and C-susceptibility of PAEC

Fig. 18

PAEC were cultured in the presence of 10 nM PMA. Cells were harvested and analysed for expression of pig CD59 and pig MCP and other cell surface markers and susceptibility to lysis by NHS





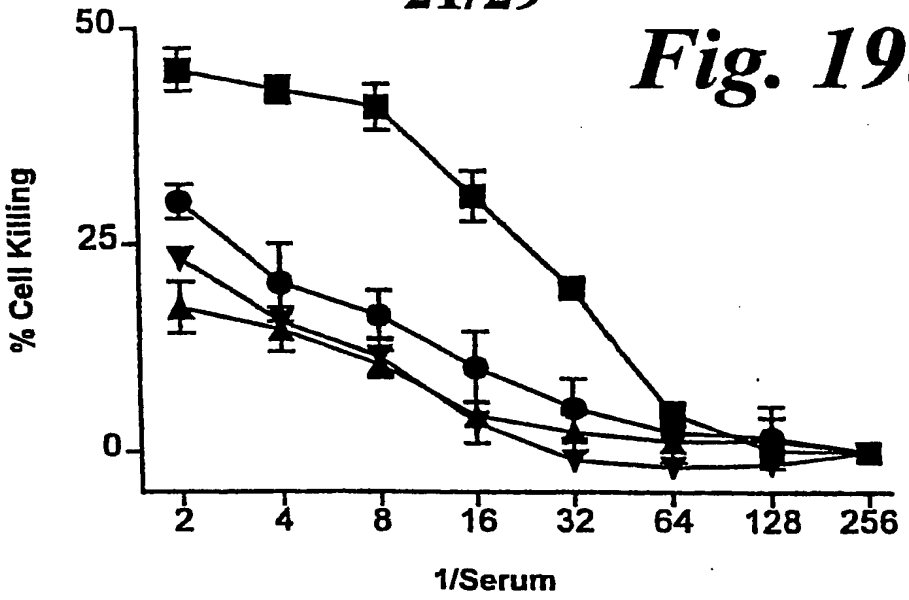


Fig. 19a

Effect Of Non-Lethal Complement Attack on the Lysis Of PAE cells PAE cells were incubated with 1/20 (▲), 1/30 (▼), 1/40 (●) or zero human serum (■) before being used in a propidium iodide cell killing assayagainst NHS. Values are means of triplicates ± SD.

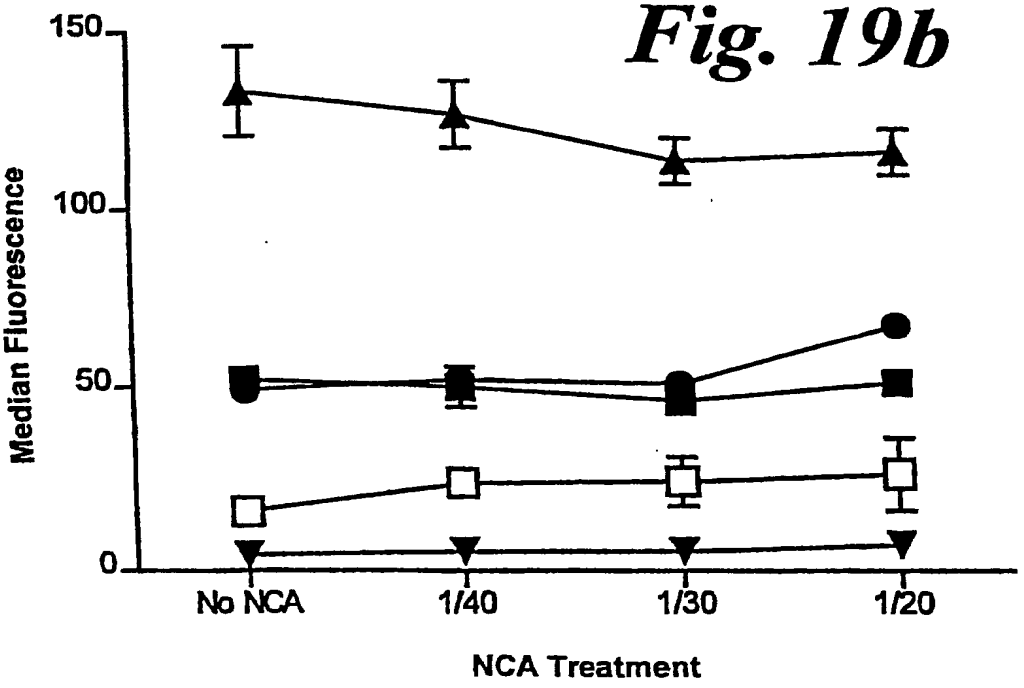
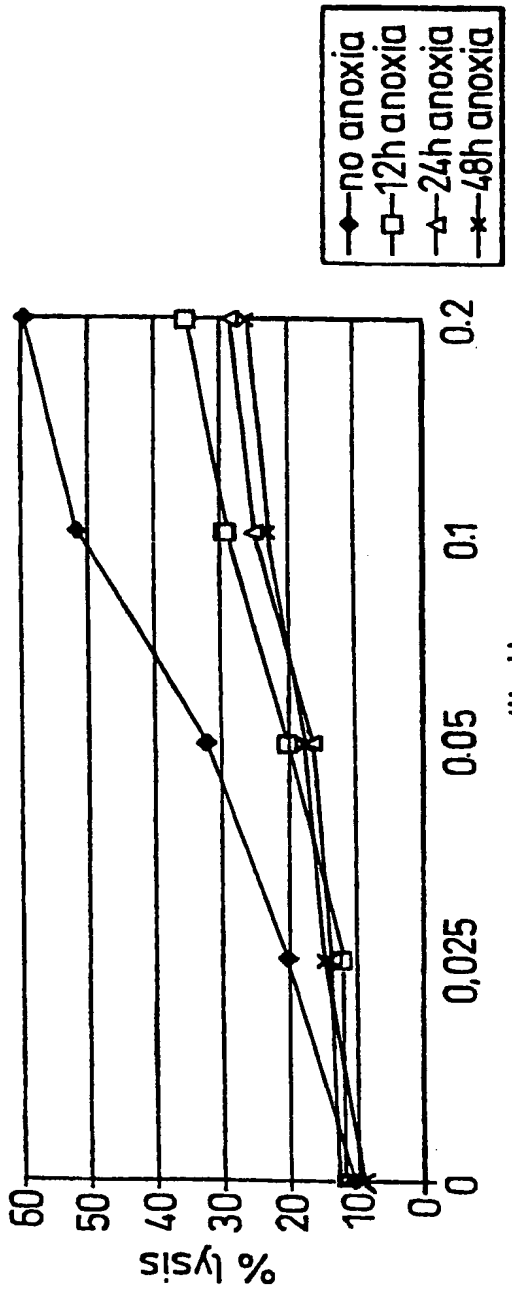


Fig. 19b

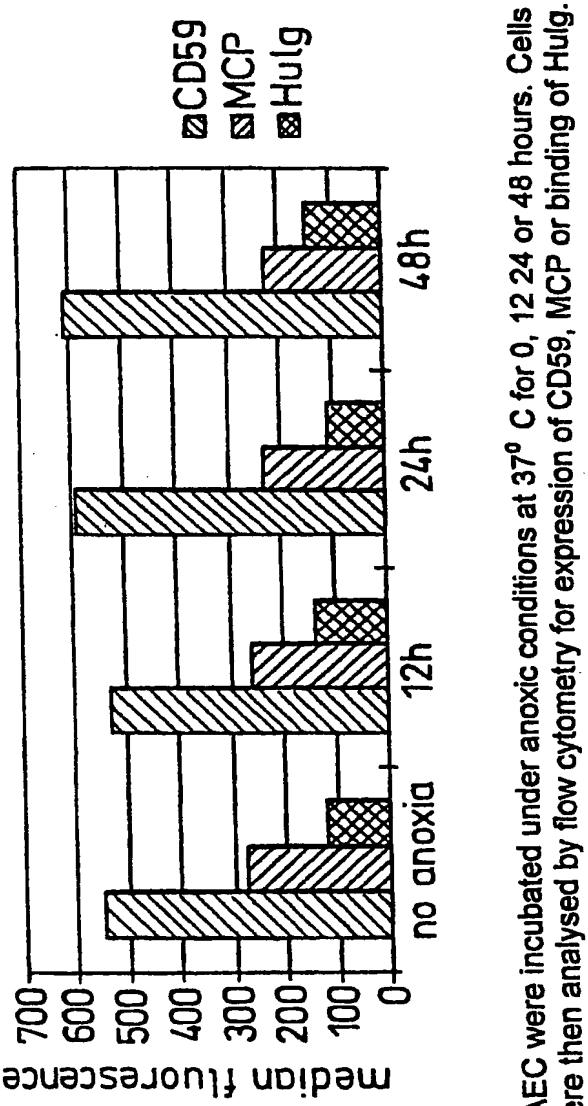
Staining of NCA Treated PAE Cells Sensitised PAE cells were incubated with different non-lethal concentrations of human serum. These cells were the then stained for MCP (■), Human IgG (●), CD59 (▲), P-selectin (total cells) (□) or P-selectin (positive staining cells) (▼) Values are means of triplicates ± SD.

Fig. 20a Effects of anoxia

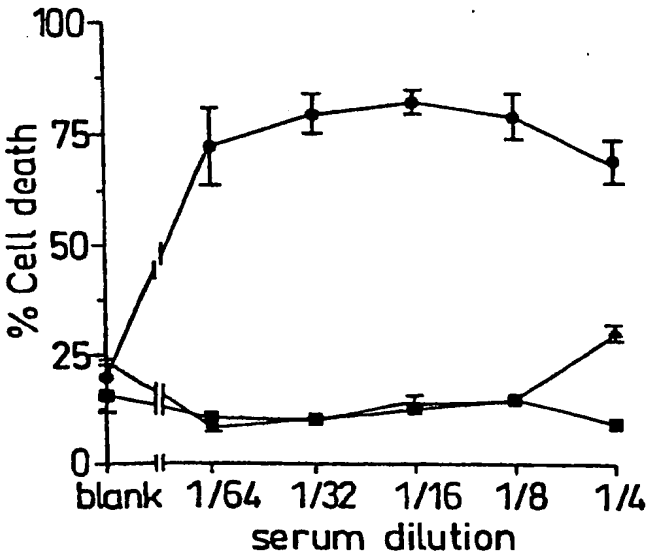


PAEC were incubated under anoxic conditions at 37°C for 0, 12 or 48 hours. Cells were then subjected to complement attack by exposing to various dilutions of human serum

Fig. 20b **Effects of anoxia**



PAEC were incubated under anoxic conditions at 37° C for 0, 12 24 or 48 hours. Cells were then analysed by flow cytometry for expression of CD59, MCP or binding of Hulg.



a: K562 cells were growth-arrested either by nutrient deprivation (triangles) or by maintaining at confluence in culture (squares). Control cells (circles) had been maintained in log growth in normal medium. Cells were then antibody sensitised and exposed to various dilutions of human serum. End-point lysis was measured at 60 min.
b: Cells growth arrest as above were stained for the various complement inhibitors and analysed on the FACScan. Open bar; control; hatched bar; confluence; solid bar; nutrient deprived. All points are mean +/- SD of triplicates.

Fig. 21a

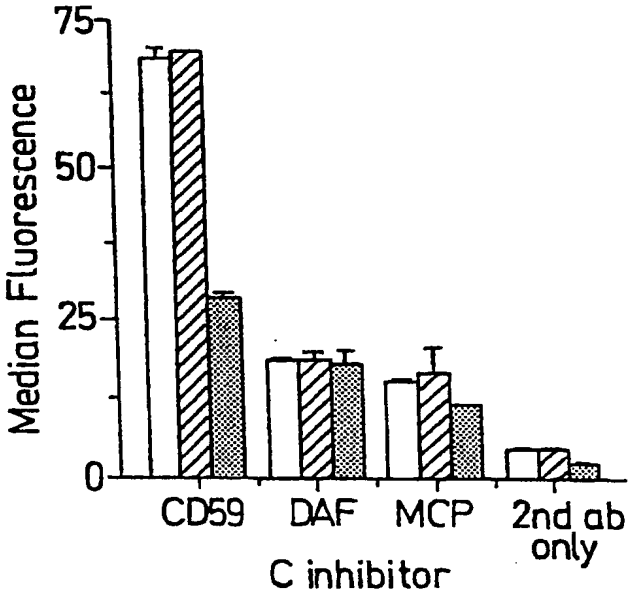


Fig. 21b

Expression of pig CD59 on pig aortic endothelial cells (PAEC) at different passages.

Cells were harvested from pig aortae and cultured. Cells were stained for pig CD59 using mAb's Mel2 and Mel3. after 1 day culturing (Primary) or after subculturing (P1-P5, appr. 4-7 days between passages).

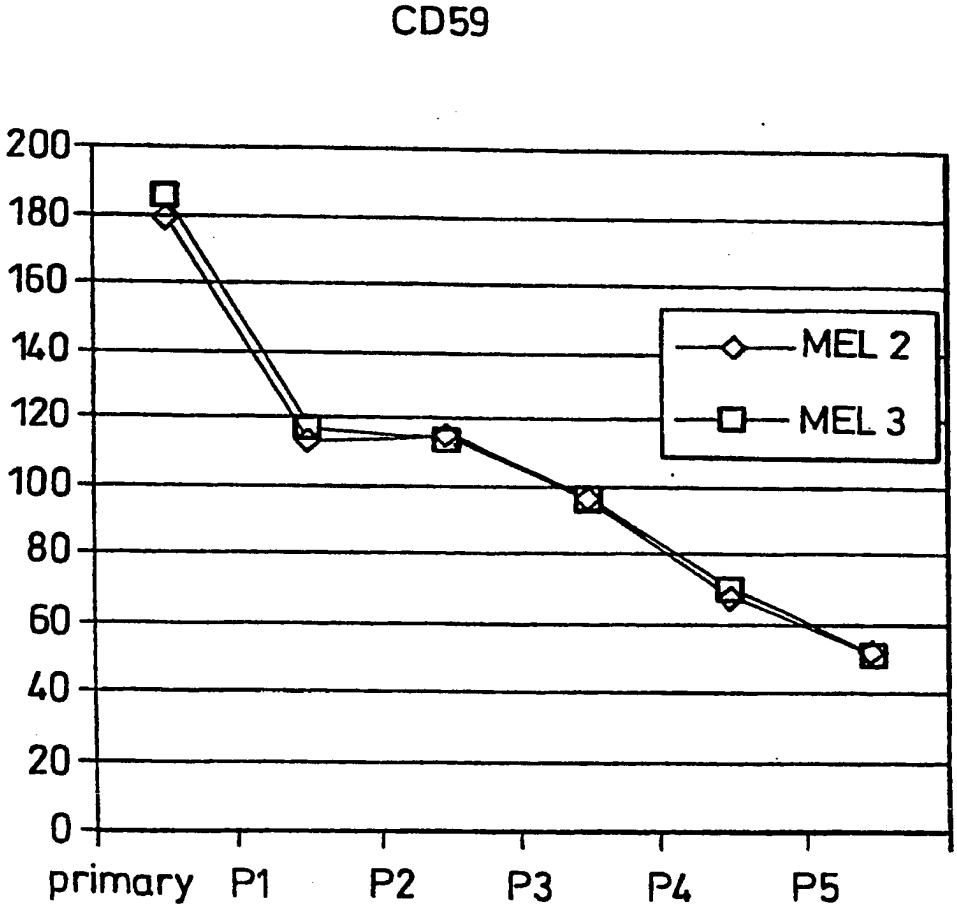


Fig. 22

Expression of pig MCP on pig aortic endothelial cells (PAEC) at different passages.

Cells were harvested from pig aortae and cultured. Cells were stained for pig CD59 using mAb's 4C8 and 1C5. after 1 day culturing (Primary) or after subculturing (P1-P5, appr. 7 days between passages).

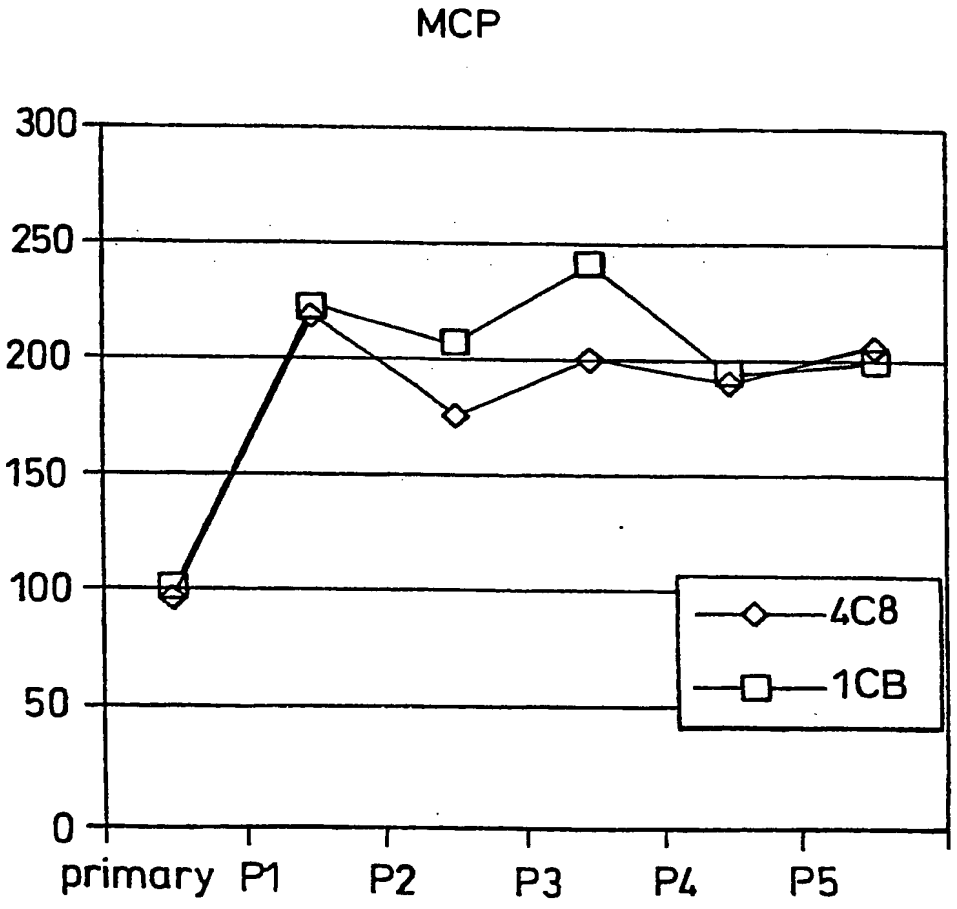
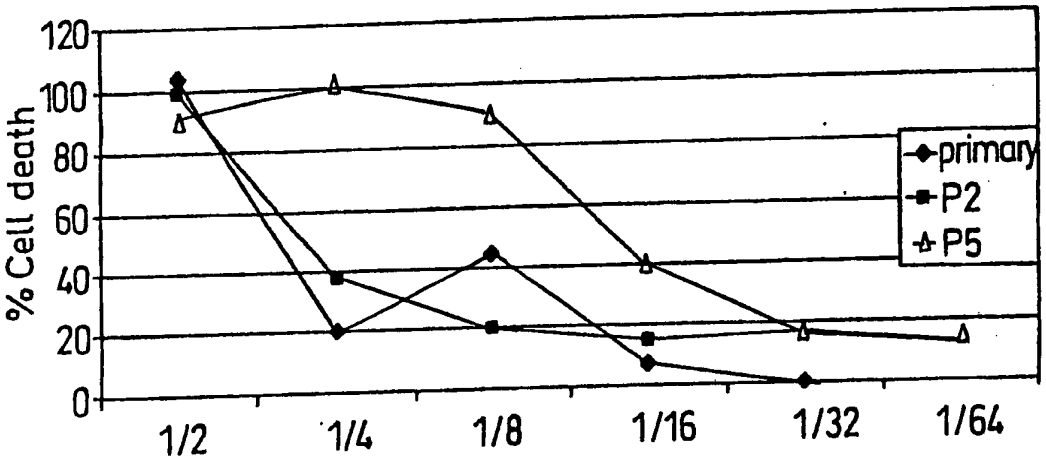


Fig. 23



C-susceptibility of pig aortic endothelial cells (PAEC) at different passages.

Cells were harvested from pig aortae and cultured. Cells assayed for C-susceptibility. after 1 day culturing (Primary) or after subculturing (P2 and P5). The cells were also analysed for the expression of CD59, MCP and binding of human Ig

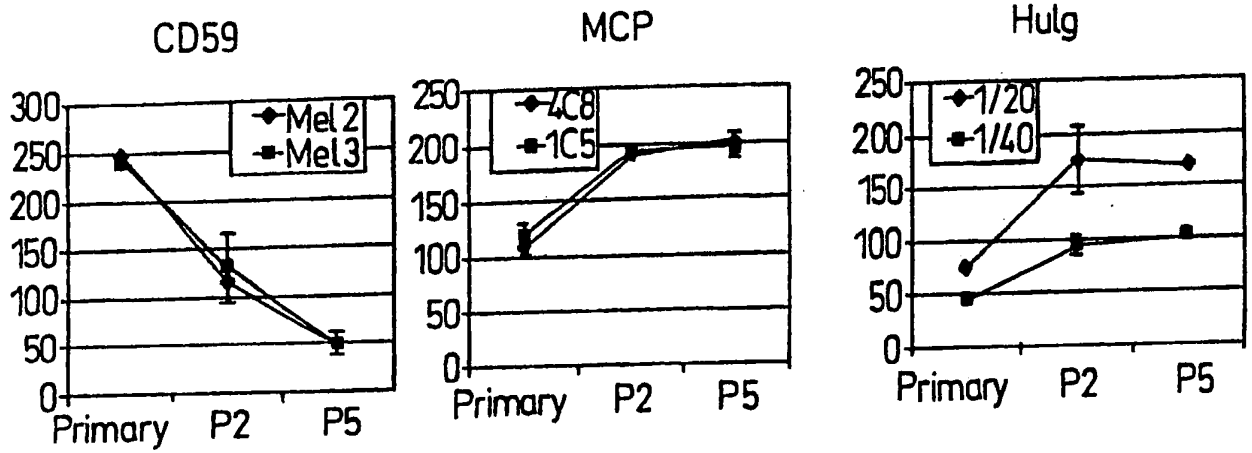


Fig. 24

Effect of blocking CD59 and MCP of C-susceptibility of PAEC.

PAEC were incubated with blocking Ab's against CD59 and MCP and C-susceptibility was assessed after challenging with HuS

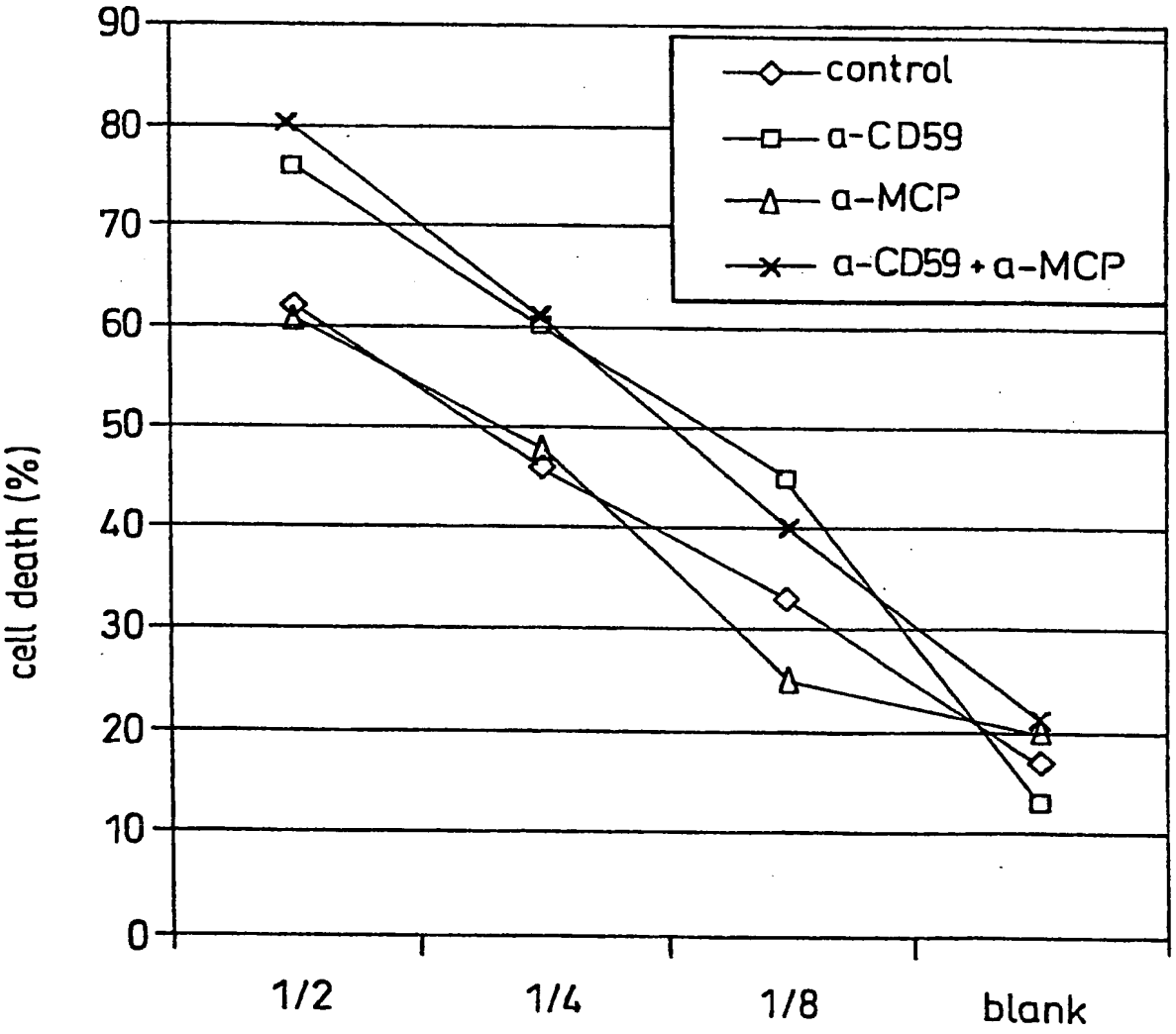
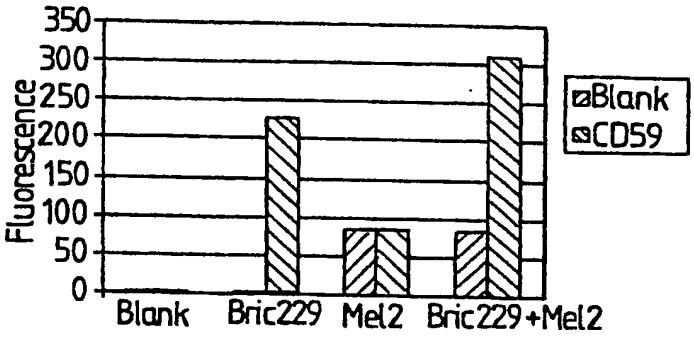


Fig. 25

Incorporation of Human CD59 into PAEC and effect of blocking of human and pig CD59 on C-susceptibility.

PAEC were incubated with 1 µg/ml CD59 for 30 min and followed by incubation with blocking antibodies against Human CD59 (Bric229) and pig CD59 (Mel2). Cells were assayed for C-susceptibility and levels of pig and human CD59



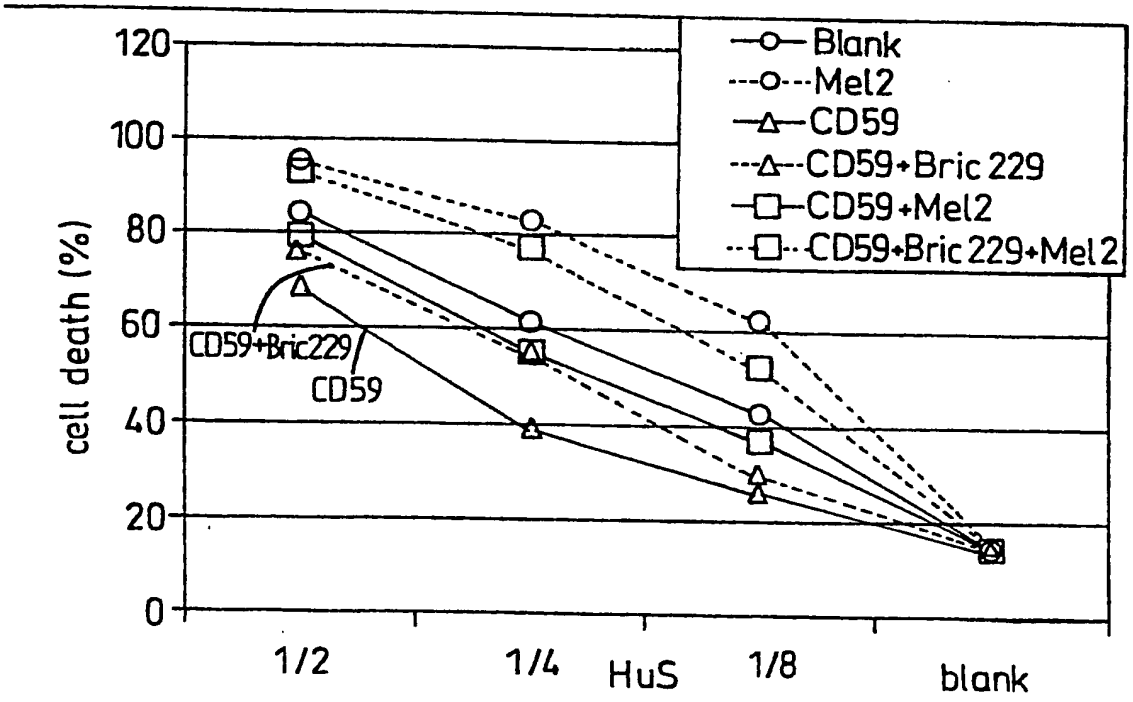


Fig. 26